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The sensitivity of *A. tonsa* to total copper ranged from 9.0 to 78.0 $\mu g\,l^{-1}$ for 72 h LC50's. Results show an inverse correlation ($P < 0.05$) between the log LC50 and adult *A. tonsa* density at the time of collection. The relationship between *A. tonsa* sensitivity to copper and the food ration (algal density/*A. tonsa* density) has been described by a quadratic function which has a correlation coefficient of 0.78. This indicates that the log LC50 increases with increasing food ration up to a point and then remains constant. A comparison of confidence intervals about the LC50's indicates greater variability in the response of field animals compared to previous studies with cultured populations. This data suggests that field populations of *A. tonsa* can be expected to exhibit a wide range of sensitivity to other pollutants. Sensitivity is strongly correlated with population density and food ration.

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THE EFFECT OF NUTRITION ON THE RESPONSE OF FIELD POPULATIONS OF THE CALANOID COPEPOD *ACARTIA TONSA* TO COPPER

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Abstract—Continuous flow toxicity tests were conducted on field populations of adult *Acartia tonsa* collected from Narragansett Bay. Potential algal food at the collection site was estimated from ATP and chlorophyll analysis. There was positive correlation ($P < 0.01$) between chlorophyll *a* and ATP. Integration of these data with quantitative zooplankton analysis established that *A. tonsa* had a significant grazing effect on algal biomass ($P < 0.05$). The natural population dynamics of *A. tonsa* during this period were assessed from microscopic analysis of tow material.

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INTRODUCTION

Zooplankton has traditionally been collected from the field for use in bioassays (Corner & Sparrow, 1956; Wisely & Blick, 1967; Lewis *et al.*, 1972). When test organisms from natural field populations are used, unpredictable variability contributes to the results due to nutritional background, population dynamics, predatory stress, and other unknown factors. Therefore, an assessment of variability is necessary before setting water quality criteria or for monitoring an effluent.

In this study, evaluation of the natural variability of a field population was measured using control survival and sensitivity to a stress. Several parameters were monitored to determine whether the observed variation was related to changes in algal food density and age structure of the population.

Recently, culture systems have been designed which provide several generations of nutritionally sound animals (Gentile *et al.*, 1974). These animals provide reproducible bioassay results with a smaller range of variability than do field animals (Sosnowski *et al.*, 1978). Statistical analysis of the variability of cultured and field populations is necessary for the interpretation and comparison of bioassay results.

MATERIALS AND METHODS

Collection techniques

Zooplankton and phytoplankton samples were taken twice a week, over a 6 month period, at a depth of 3 m at a station located in the west passage of lower Narragansett Bay, Rhode Island.

Zooplankton was collected by towing 300 mm, metered Bongo nets fitted with 153 μm Nitex netting for 3 min at a speed $< 0.5\ m\,s^{-1}$. The starboard (right hand net) sample was diluted fivefold with ambient seawater and then returned directly to the laboratory in an insulated container to provide test animals for a bioassay. Sub-samples of 250 ml were then immediately transferred to 90 × 100 mm Pyrex crystallizing dishes and diluted to ~2000 ml with filtered (0.45 μm) ambient seawater. Two 20 ml aliquots from the port (left hand net) sample were preserved in 10% formalin for qualitative–quantitative counts. From these counts, the percent composition of total zooplankton and the age distribution of *Acartia tonsa*, the dominant calanoid copepod, were determined.

Phytoplankton samples were collected with a Nansen bottle. Aliquots of the sample were immediately prepared for adenosine triphosphate (ATP) and chlorophyll *a* measurements.

Biomass determination

Algal levels were determined through a comparison of ATP and chlorophyll *a* (*in vivo* and *in vitro*). Non-filtered extraction of ATP was accomplished by injecting 0.2 ml of sample into 5 ml of boiling Tris buffer. The 5 replicate samples were then frozen and stored for later analysis (Cheer *et al.*, 1974). Chlorophyll *a in vivo* and *in vitro* were analyzed fluorometrically in accordance with the methods of Holm-Hansen *et al.* (1975). *In vivo* chlorophyll *a* was read directly for three replicates on a Turner III fluorometer using a high sensitivity door and an R-136 photomultiplier tube. Samples for *in vitro* chlorophyll *a* were obtained by filtering three replicate 25 ml samples with $MgCO_3$ through 0.45 μm glass-fiber filters which were frozen over silica gel. Samples were subsequently extracted with cold 90% acetone for analysis.

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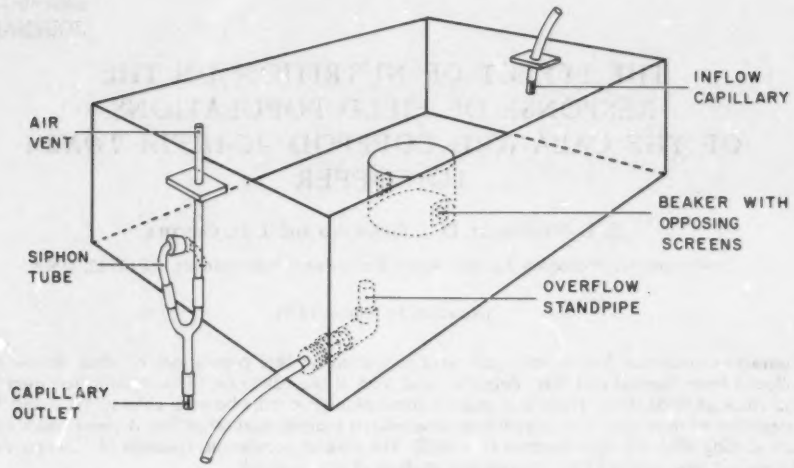

Fig. 1. Siphon-flush system illustrated as modified.

Bioassay procedure

Continuous flow bioassays were performed in filtered seawater maintained at a temperature of 20 ± 2°C and 30‰ salinity. Light was maintained at 1000 lux on a 12L:12D cycle.

Three replicates of 15 animals each were employed for each of 5 toxicant concentrations and a control. The assay was continued for 96 h with mortalities removed and recorded daily (Gentile *et al.*, 1974; Standard Methods, 1976).

Toxicant solutions were prepared at one hundred times the final desired concentration. The stock solutions were metered at a constant rate and proportionally diluted (1:100) with filtered seawater. The flow rate of dilution water was controlled with capillary tubing.

The continuous flow assay system used was a modification of the siphon-flush system by Buchanan *et al.* (1975) redesigned to accommodate the calanoid copepods exposed in this study. Using glass capillary tubing, the inflow rate was set at 20 ml min^{-1}. The outflow, when siphoning, discharged at a rate of 100 ml min^{-1}. This allowed a 50% exchange of water in the exposure vessels every 4 h. Nalgene beakers (100 ml TPX) and Plexiglas boxes (350 × 275 × 130 mm) were used to avoid positive or negative contamination (absorption or leaching) of metals in the test waters. Nitex screen (70 μm) covered two holes 10 mm in diameter drilled on opposite sides of the beaker, 20 mm from the bottom. This allowed occlusion of the holes by the thumb and forefinger, covered by finger cots, so that the beaker could be lifted from the box without changing the liquid level. This prevented impingement of animals against the screen caused by rapid loss of water. The beaker was then placed into a 100 ml Carolina dish containing seawater at the same level as that in the beaker. The beaker could then be examined under the dissecting microscope without loss of liquid. Beakers are always checked from low to high concentrations to prevent contamination.

Also, an improved siphon-flush mechanism was devised to prevent air locks (Fig. 1). The siphon was a glass tube (i.d. 5 mm) bent into a U shape and inserted through a silicone rubber stopper (size 2) which was inserted into a hole 20 mm in diameter and centered 55 mm above the floor of the box. The inside portion of the siphon tube was 30 mm above the floor of the box. The outside portion was connected with silicone rubber tubing to one arm of a glass-U (i.d. 5 mm). Connected to the other arm of the glass-U was a section of glass tube (i.d. 5 mm) which reached to the top of the box and served as an air vent. This device prevented air locks inherent with slow flows. Capillary tubing, controlling the rate of outflow was connected to the bottom arm of the glass-U with silicone rubber tubing. An overflow standpipe of 12.5 mm diameter PVC was installed at a height 15 mm below the top of the beakers to prevent their flooding and loss of animals in the event of siphon malfunction.

Copper [primary standard for atomic absorption; Cu $(NO_3)_2$] was used as the toxicant source in all tests. Exposure levels were determined from results of range-finding tests and were based on progressive bisection of intervals on a logarithmic scale. Copper was selected because of its predictable response curve and our analytical capability to monitor total copper in seawater at the levels used. Dose response curves were examined by probit analyses to determine 72 h LC50's (lethal concentration for 50% of tests animals) and the 95% confidence limits. Rates of response between populations were then compared by determining whether probit slopes were parallel. Differences in sensitivity were determined by comparing differences in LC50's using tests for relative potency (Finney, 1971). Regression analysis was used to compare phytoplankton measurement techniques and phytoplankton–zooplankton relationships.

RESULTS

Seasonal patterns of chlorophyll *a* and ATP were used as estimates of the algal biomass potentially available to zooplankton populations. A peak occurred during August as the result of reduced grazing pressure by zooplankton which were being heavily predated by ctenophores during that period. The positive correlations among the two chlorophyll *a* measurements and ATP ($P < 0.01$) indicate that either of these techniques could be used singly to provide an estimate of algal food. ATP measurements as employed in these studies, while potentially measuring all living material, basically reflect algal biomass. The grazing effect of the zooplankton on

algal biomass was illustrated by an inverse correlation ($P < 0.05$) between total *A. tonsa* density and extracted chlorophyll *a*.

Collection, capture, and transfer of test animals from field populations to the bioassay system apparently stressed the organisms excessively. Control mortality at 96 h was often in excess of 25% which we feel may bias the results even though statistical analysis takes this into account. Therefore, all bioassay data are for 72 h exposure periods for which control mortality was $\leq 15\%$. It is recommended that control mortality be used as a guide in selecting exposure periods for field populations.

The relationships between algal density (μg chlorophyll *a* l^{-1}), zooplankton density/m^3, *Acartia tonsa* density/m^3 and toxicological data (72 h LC50 *Acartia tonsa*) are presented in Fig. 2.

During the interval 1–16 October it was observed that the LC50 values decreased in a manner similar to the phytoplankton density, which in turn was responding to the rapid rise in the zooplankton population. On 1 October there was a condition of low zooplankton density and high food levels which offered a high food ration (food/animal), and thus potentially optimum nutritional conditions. During the next two weeks the situation reversed itself and by 16 October there were maximum zooplankton densities and minimal food. This indicates that the increased sensitivity (decreasing LC50 values) of *Acartia tonsa* was directly related to the interaction of algal and zooplankton density changes, expressed in terms of food ration per animal.

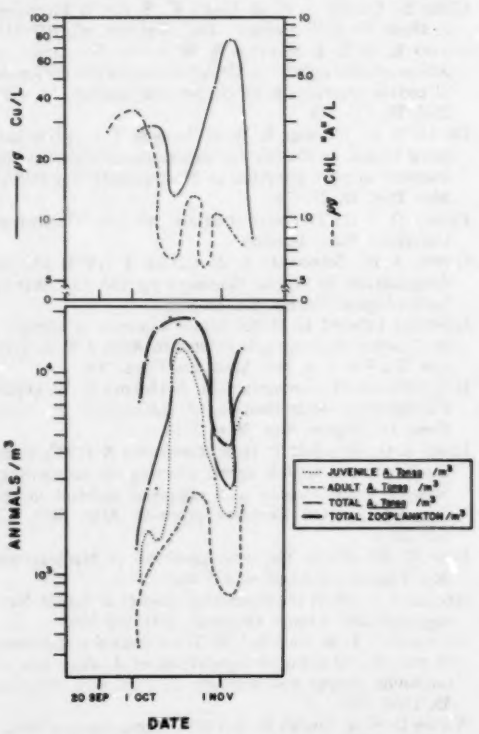

Fig. 2. Relationships between algal density (μg chlorophyll *a* l^{-1}), zooplankton density/m^3, *Acartia tonsa* density/m^3, and toxicological data (72 h LC50 *Acartia tonsa*).

During the interval 16 October–3 November there was little observed change in algal density, but a precipitous decrease in zooplankton density was noted. There are two possible explanations: over-grazing of the food supply and predation. The chlorophyll data suggest that there was still adequate food to support the zooplankton densities present. Further, the amplitude of change appeared to be much greater and more extreme for the zooplankton than phytoplankton. The change in zooplankton density was apparently the result of predation since the total zooplankton population, including herbivores (*Acartia tonsa*) and carnivores, was affected. Also, both age classes of *Acartia* were affected simultaneously (Fig. 2). Had food become limiting, a shift in age-structure toward a higher percentage of adults as compared with juveniles would have been observed because the amount of food available is directly related to the number of eggs released (Wilson & Parrish, 1971).

Toxicological responses showed a sharp decrease in copper sensitivity (increase in LC50) from 16 to 27 October. As algal densities remained relatively constant, zooplankton densities decreased, and the food ration again was favorable as reflected in the LC50 values. If predation pressure was released between 27 October and 1 November, the remaining *A. tonsa*, initially having had a high food ration, would produce larger numbers of eggs (Wilson & Parrish, 1971). At a temperature of 14°–15°C, these eggs would be expected to hatch within 36 h and appear as juveniles 5–8 days later. There was a rapid rise in zooplankton density during this period (Fig. 2). This greatly reduced the food ration because the algal density was constant, while the zooplankton density increased 5-fold. Under these conditions, one would expect these organisms to be more sensitive to toxicants. The LC50's for organisms collected on 10 November was, in fact, very low (9 μg Cu^{2+} l^{-1}). There was a significant inverse correlation ($P < 0.05$) between the log LC50 and adult *A. tonsa* density.

DISCUSSION

The comparison of toxicological data derived from laboratory populations to data from similar field populations is difficult. One must have an estimate of the toxicological variability of temporally separate natural populations. This is most easily done with a holoplankter such as *A. tonsa* which has an approximately 20 day life cycle at 18°C. These studies were originally designed to include weekly flow-through tests over the whole seasonal cycle of *A. tonsa* (May–November). *A. tonsa* did not develop until late July and then was immediately grazed by ctenophores.

Population densities adequate for testing were not available until late September.

The data (Fig. 2) indicate a wide range of sensitivity of *A. tonsa* to copper. This range differs from previous studies on laboratory cultured *A. tonsa* which produced 96 h LC 50's of 17–55 $\mu g\,l^{-1}$. Thus, the wide range in field *A. tonsa* responses to copper, coupled with higher natural (control) mortality, indicates a greater variability than laboratory populations. Additional evidence to support this hypothesis emerges when the confidence intervals for assays performed in this study are compared with those from laboratory studies. The mean variability of field populations above the LC50 value was 61% as compared with 38% for cultured populations. The mean variabilities below the LC50 were 48 and 36% for field and cultured populations respectively. The toxicological responses of the six field populations were examined statistically using analysis of variance. The differences between these populations were highly significant ($P < 0.01$). Therefore, the range of responses to copper obtained from populations collected at different times truly reflected the biological condition of the organisms.

The toxicological data from this study was compared with potential algal food, population patterns of *A. tonsa* and food ration. The purposes of these comparisons were to determine what relationships existed between these factors and whether they could be used to explain the observed variance. Comparison of *A. tonsa* density with algal biomass indicates a strongly negative relationship (grazing) that was statistically significant ($P < 0.05$). The log LC50's were inversely correlated ($P < 0.05$) with *A. tonsa* density. Thus, as the *A. tonsa* population increased in density their sensitivity also increased, since a decrease in LC50 reflects an increase in sensitivity. Because increasing population density is also causing a decrease in potential algal biomass due to grazing, we compared log LC50 with changes in food ration (food/*A. tonsa*). Unlike the other relationships which were linear, this relationship was best described by a quadratic function ($y = 0.93 + 52.68x - 852x^2$; $R = 0.78$). This relationship reveals that as the food per animal increases, the log LC50 also increases to a maximum. After reaching a certain food ration, its continued increase has no further effect on the sensitivity of the *A. tonsa*. This is the simplest model and by itself does not imply a causal relationship. Further laboratory studies are necessary to establish and quantify the significance of food ration to toxicological sensitivity. The implication is clear, however, that variable sensitivity of natural populations of the herbiverous holoplankter *A. tonsa* is related to food ration.

The variable sensitivity of natural *A. tonsa* populations presents a strong case for taking a conservative posture in setting marine water quality standards. Ambient copper levels in the marine environment range from 1.5 to 3.0 $\mu g\,l^{-1}$ (Goldberg, 1965). Near shore waters receiving waste water and runoff often reach levels $\geqslant 15\,\mu g\,l^{-1}$. Acute toxicity studies with laboratory cultured populations of *A. tonsa* result in a 96 h mean LC50 value of 17 $\mu g\,l^{-1}$ with a lower confidence interval of 9 μg l. This study indicates that the 72 h mean LC50 can be as low as 8.0 $\mu g\,l^{-1}$ with a lower confidence interval of 5.5. This indicates that one of the most important and ubiquitous neritic calanoids is acutely sensitive to copper at concentrations that are approaching ambient. There is a strong probability that sub-lethal effects are occurring at or slightly above ambient levels. The significance of this field study is two fold: it verifies previous laboratory studies on cultured populations of *A. tonsa*; and it demonstrates the range of toxicological variability one might expect from holoplankton grazers.

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